IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		§	
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	YOSEPH SHAALTIEL	§	
		§	
Serial No.:	10/784,295	§	
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Filed:	February 24, 2004	§	Group Art Unit: 1744
		§	
For:	CELL/TISSUE CULTURING DEVICE, SYSTEM AND METHOD	§	
		§	Attorney
		§	Docket: **27557**
Examiner: BEISNER, William H.		§	

Commissioner for Patents
POBox 1450
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DECLARATION OF DR. YOSEPH SHAALTIEL UNDER 37 CFR §1.132

I, Yoseph Shaaltiel, am the Chief scientist and E.V.P. R&D of Protalix Ltd. at Carmiel, Israel. My professional specialization is in the field of Plant Genetics and Biochemistry, particularly with regard to plant cell culture and industrial applications of plant cell cultures. I have attached a copy of my curriculum vitae with some of the most recent publications.

I am the inventor of the present invention. I have read the present application and the new and amended claims, as well as the Office Action from the Examiner and the accompanying references. In support of the accompanying Response to this Office Action, I set forth below some important historical and experimental data which demonstrates some of the unique characteristics of the claimed invention.

In this Official action, the Examiner has rejected claims 99-102, 104, 108-114 and 116-121 under 35 U.S.C. § 102(b), asserting anticipation by US Patent No. 5,534,417 to Arad et al., claims 103, 013, 106, 107 and 115 under 35 U.S.C. § 103(a), asserting obviousness over US Patent No. 5,534,417 to Arad et al. in view of Kalfon (EP 343 885), or Hitzman (US 4,519,984), or Kobayashi (US 5,565,015) or Whitney (GB 2 202 549). The results of the investigation of optimal parameters for the design

and operation of low-cost, disposable bioreactors for culturing of plant cells described hereinbelow are consistent with the results disclosed in the instant specification, indicating that the claimed bioreactors, systems and methods for culturing recombinant plant cells for production of recombinant proteins are significantly distinguished from the methods and devices for growing algae disclosed by Arad et al.

Claim 99-121 relate to a system for axenic culture of plant cells or tissue, using a disposable bioreactor having a generally cylindrical geometry, with air inlet pipes of defined dimensions to provide bubbles with correct bubble size to effectively mix and aerate the plant cell culture without causing shear and cell damage. Culture conditions resulting in efficient culture growth and high yields of recombinant proteins are described in detail in the present application.

As an inventor of the present application, I either performed myself or caused to be performed numerous experiments to assess the feasibility of using plant cell bioreactors to produce recombinant mammalian proteins. The development process of a bioreactor for plant cells started early 1994 once the company realized that conventional reactors are non economical for the purpose of mass production of proteins from plant cells, in terms of capital investment per production facility.

Although effective methods for gene transfer in plant cells were commonly available at the time, the main challenges that we faced were to develop a reactor that will enable axenic culturing of viable plant cell cultures for a period of over several weeks, allowing harvesting of a pharmaceutical product in significant amounts. In addition, in order to meet industrial-scale needs, the designed bioreactor needed to be biologically efficient i.e. present a growth rate which is as similar as possible to lab scale growth. Further, economic efficiency required the reactor to be competitive in cost with the microbial and mammalian protein and metabolite production systems currently operating and in development in the industry.

Since plant cells contain a rigid cell wall which is susceptible to shear in culture, meeting the challenges of industrial scale plant cell culture required a reactor with an efficient mixing system operating without vanes or impellers.

In addition, meeting the challenge of economically competitive production of recombinant proteins in industrial scale plant cell culture required cost-effective production using simple materials for the bioreactor structure and a minimum of control equipment.

Systems suitable for growth of algae, known at the time, were not relevant since, in their natural environment, algae grow in cell suspension and are thus evolutionarily suited for growth in liquid media, while plant cell suspension culture is an artificial, man-made environment, imposing unique limitations and posing previously unencountered hazards to the growing plant cell. Most significant were the need for gentle but effective mixing (to reduce shear forces), on the one hand, and providing adequate aeration on the other hand.

In order to provide an efficient plant cell bioreactor, our first efforts were directed to the development of a small reactor made of polyethylene in which the mixing of the cell culture is caused by air bubbles only, and growth conditions are monitored at the beginning and at the end of a growth cycle.

The development of an efficient plant cell bioreactor for culturing viable cells in axenic conditions continued for more than two years (January 1994- October 1996).

During this time period, experiments were performed to evaluate a number of bioreactor parameters and culture conditions. Cells were cultured for a duration of 1-14 days, and 3 or more duplications were conducted in order to assure statistical significance. It is therefore estimated that at least 25 experiments with 75 bioreactor prototypes were completed each year.

Several parameters were assessed after each experiment, including packed cell volume (calculated from the height of settled cells in the bioreactor, following cessation of aeration), wet cell weight, dry cell weight and cell viability.

The first element that was experimentally evaluated was the bioreactor shape. Numerous cylindrical shape types were assessed, with emphasis on the design of the reactor bottom. Among the designs tested were flat, conical and triangular-shaped bottoms. Culturing began with 10% packed cell volume, and was monitored for up to 2 weeks.

Evaluating viability and culture growth, it was found that a shallow frusta conical shaped bottom was the least harmful to the plant cells in culture and further bioreactor development used this design.

In all the above trials air was supplied via an air sparger, which is the common industrial practice for air bubbling into bioreactors. Experiments were conducted with air spargers from 3 different types, 2 types of sinter-glass spargers with varying pore sizes and 1 mineral rock sparger. All spargers had pore sized under 1mm.

Using different types of air spargers, no cell growth was observed under any of the culture conditions tested during the first stages of development. Moreover, in all cases an obvious decline in viability of the cell culture was observed following 1-5 days of culturing, with the packed cell volume deteriorating to less than 10%.

Results indicated that air outlets with pore sized under 1mm produce air bubbles which are destructive to the plant cell. It was therefore concluded that the small bubbles produce high shear force that kill the plant cells and the negative balance between cell division and cell death outcomes was a declining growth rate.

Experiments during the second stage of investigation were directed to studying alternative methods of aeration. The demand for sterility in this type of culturing caused unpredictable results using conventional means of aeration known at the time. In order to find a balance between shear forces, aeration and mixing of the culture, different sizes and positioning of air inlet orifices, through which air was bubbled, were evaluated, producing different size ranges of bubbles. Varying this parameter was found to be effective, until an optimal growth rate of the plant cell culture was reached. Different designs of air inlets were evaluated, including a silicone pipe that was placed in the reactor from the top, the orifice reaching down to near the bottom of the reactor. However, the result with this design quickly showed that the bubbling caused the pipe to move within the reactor, tending to float upwards. Different attempts were tried in order to keep the pipe at the bottom, while maintaining sterility, including welding of the pipe to the reactor wall- and different types of weights to keep the tube positioned near the bottom of the reactor. None of our efforts were successful, with all of the designs disrupting the continuity and smoothness of the reactor wall, causing turbulence and/or compromising the mixing efficiency of the cells, resulting in reduction in culture efficiency, cell sedimentation, cell death and decay of culture. In addition, basic cost of the bioreactor was increased, due to the sterilization requirements of the introduced elements. In order to address this issue, we settled on integral air openings placed directly at or near the bottom end of the reactor, using a rigid insert. Optimal bubble size, determined during the trials, was achieved with air inlets (orifices) of at least 4mm.

Once the location, shape of orifice and desired bubble size were established, reactors constructed of flexible and rigid, transparent and non transparent materials were assessed. Results indicated that each of the different combinations had

limitations either in growth rate or in cost of handling. Following these experiments, it was ceterminded that the use of a non-rigid transparent bioreactor with air orifices of 4mm diameter, located at or near the reactor's bottom end led to superior biological parameters (quality of the culture and it's recombinant products), and greatly improved economic efficiency of production.

As a person signing below, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Application or any patent issued thereon.

June 29, 2008

Dr. Yoseph Shaaltiel

Enc.: CV of Dr. Yoseph Shaaltiel

Curriculum Vitae

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Date & Place of Birth: March 17, 1953, Israel

Professional Experience:

2006-present	Executive Vice President, Research and Development-***Protalix Biotherapeutics*** *(former: Metabogal Ltd.)*
1996-2006	Director of Research and Development - ***Metabogal Ltd.***
1993-1996	General Manager and Chief Scientist - ***Metabogal Ltd.***
1989-1993	Research associate - ***Migal Technological Center***, Galilee.
1988-1989	Postdoctoral Scholar, B.A.Zilinskas Laboratory, Dept. Biochemistry & Microbiology, ***Rutgers University, NJ.***
1987-1988	Postdoctoral Scholar, B.N.Ames Laboratory, Dept. Biochemistry, ***University of California,*** Berkely, CA
1982-1987	Research Student, Plant Genetics Dept., ***Weizmann Institute of Science,*** Rehovot, Israel.
1981-1982	Deputy Head, Biology Dept., ***NBC Center, IDF.***
1980-1981	Biochemist, ***Makor Chemicals Ltd., Jerusalem.***
1978-1980	Research Assistant, Dept. Biology, ***Ben-Gurion University.***
1977-1978	Undergraduate research technician, Dept. Biology, ***Ben-Gurion University, Beer Sheva, Israel***

Teaching Experience:

1993-1995	***Clinical Biochemistry*** for Bachelors degree in life scienses, Emek Hyarden College, under Bar Ilan Universty supervision.
1993-1995	***Biochemistry*** for Bachelors degree in life scinses, Emek Hyarden College, under Bar Ilan universty supervision.
1993-1998	***Chemistry*** course for bachelors dgree in life scinses, Emek Hyarden College, under Bar Ilan universty supervision.
1990-1994	***Plant Tissue Culture*** course, Biotechnological program, Tel-Hay college.
1990-1992	***Scientific director*** of youth scientific summer camp.
1989-1993	***Projects Scientific Director*** for high school students.
1989-1992	***Projects Scientific Director*** for biotechnological students, Tel-Hay college
1989-1990	***Inorganic Chemistry*** course, Hebrew University, Faculty of Agriculture. First year course.
1980	***Teaching assistant,Plant Physiology***, Ben-Gurion University.

Education:

1982-1987	***Ph.D. in Plant Biochemistry, Dept. of Plant Genetics, Weizmann Institute of Science, Rehovot.*** Thesis title: Physiology, biochemistry and genetics of resistance to paraquat and other oxidant generating xenobiotics in Conyza and other plant species Supervisor: Prof. Jonathan Gressel.
1983	***ICRO(Special course) , Stockholm.*** The metabolism of xenobiotics and its relationship to chemical carcinogenesis.
1979-1982	***M.Sc. in Agriculture, The Hebrew University, Rehovot*** Thesis title: Qualitative determination of polygal acturonase iso-enzymes in different tomato genotypes Supervisors: Dr. Y. Mizrachi and Prof. N. Kedar.
1975 – 1978	***B.Sc. in Biology, Ben Gurion University, Beersheba.***

Fellowships

May-April 1986	British Council Fellowship for research at University of Bath, in laboratory of Dr. A. D
1984-1985	Sephardi Community Fellowship for research at the Weizmann Institute of Science.
June 1983	ICRO fellowship for course at Arrhenius Laboratory,. Biochemistry Dept Stockholm University,.

Scientific Publications:

1. **Shaaltiel, Y.**, Bartfeld, D., Hashmueli, S., Baum, G., Brill-Almon, E., Galili, G., Dym O., Boldin-Adamsky SA., Silman, I., Sussman, JL., Futerman, AH. And Aviezer, D. **2007.** *"Production of Glucocerebrosidase with terminal mannose glycans for enzyme replacement therapy of Gaucher disease using a plant-cell system".* Plant Biotec J 5:570-590.

2. Yehuda, Y., Goldway, M., Gutter, B., Michael, A., Godfried, Y., **Shaaltiel, Y.**, Levi, B.Z. and Pitcovski, J. **2000.** *" Transfer of antibodies elicited by baculovirus- derived VP2 of very virulent IBDV strain to progeny of commercial breeding chickens".* Avian Pathology 29:13-19.

3. Pitcovski, J., Goldberg, D., Levi, B.Z., Di-Castro, D., Azriel, A., Krispel, S., Maray, T. and **Shaaltiel, Y. 1998.** *"Coding region of segment A sequence of a very virulent isolate of IBDV - comparison with isolates from different countries and virulence".* Avian Diseases 42: 497-506.

4. Pitcovski, J., Di-Castro, D., **Shaaltiel, Y.**, Azriel, A., Gutter, B., Yarkoni, E., Michael, A., Krispel, S. and Levi, B.Z. **1996.** *"Insect cell-derived VP2 of Infectious Bursal Disease confers protection against the disease in chickens".* Avian Diseases 40:753-761.

5. Gressel, J., **Shaaltiel, Y.**, Sharon, A., Amsalem, Z. **1992.** *" Biorational in vitro screening for herbicide synergists".* In: Herbicide bioassay. J.C. Strebig and P. Kudsk, Eds. CRC Press, Boca Raton.

6. Jansen, M.A.K., Malan, C., **Shaaltiel, Y.** and Gressel, J. **1989.** Mode of Evolved photooxidant resistance to herbicides and xenobiotics. Z. Naturforsch 45c:463-469.

7. Jansen, M.A.K., **Shaaltiel, Y.**, Kazzes, D., Malkin S. and Gressel, J. **1989.** *" Increased tolerance to photoinhibitory light in paraquat-resistant Conyza bonariensis measured by photoacoustic spectroscopy and 14CO2-fixation".* Plant Physiol., 91:1174-1178.

8. Gressel, J. **and Shaaltiel, Y. 1988.** *" Biorational herbicide synergists".* In: Biotechnology for Crop Protection P.A. Hedin and J.J. Menn, Eds. Amer. Chem. Soc. Symp. Series, 379, pp.4-24.

9. Gaba, V., Cohen, N., **Shaaltiel, Y.**, Ben-Amotz, A. and Gressel, J. 1988. *" Light-requiring acifluorfen action in thabsence of bulk photosynthetic pigments".* Pest. Biochem. Physiol., 31:1-12.

10. **Shaaltiel, Y.**, Glazer, A., Bocion, P.F. and Gressel, J. **1988**. *"Cross tolerance to herbicidal and environmental oxidants of plant-biotypes tolerant to paraquat, sulfur-dioxide and ozone"*. Pest. Biochem. Physiol., 31:13-23.

11. **Shaaltiel, Y.**, Chua, N-H., Gepstein, S. and Gressel, J. **1988**. *"Dominant pleiotropy controls enzymes co-segregating with para-quat resistance in Conzya bonariensis"*. Theoretica & Applied Genetics, 75:850-856.

12. **Shaaltiel, Y.** and Gressel, J. **1987**. *"Biochemical analysis of paraquat resistance in Conzya leads to pinpointing synergists for oxidant generating herbicides"*. Proc. Sixth Int. Cong. of Pesticide Chemistry. Blackwell Scientific Publications, London.

13. **Shaaltiel, Y** and Gressel, J. **1987**. *"Kinetic analysis of resistence to paraquat in Conzya: evidence that paraquat transiently inhibits leaf chloroplast reactions in resistant plants"*. Plant Physiol., 85:869-871.

14. **Shaaltiel, Y.** and Gressel, J. **1986**. *"Multi enzyme radical detoxifying syatem correlated with paraquat tolerance in Conzya bonariensis"*. Pest Biochem. Physiol., 26:22-28.

Abstracts and Presentations

Y. Shaaltiel, 2008 • *"Plant Cell Culture as a Pharmaceutical Protein Expression System and plant made Glucocerebrosidase"*. EU Pharma Law and Regulation conference London,.

Y. Shaaltiel, 2008 *"Phase II CMC Data for Plant Cell-Made rGlucocerebrosidase" 5th International BioProcess Technology, Europe • • Amsterdam, The Netherlands*

David Aviezer, Einat Almon-Brill, **Y. Shaaltiel**, Gadi Galili, Raul Chertkoff Sharon Hashmueli,,Tony Futerman Eithan Galun, Ari Zimran ***2008***. *Novel Enzyme Replacement Therapy (ERT) for Gaucher Disease: On Going Phase III Clinical Trial with a Recombinant Human Glucocerebrosidase (prGCD) Expressed in Plant Cells WORLD Lysosomal Research Network Annual Symposium February, Las Vegas, NV, USA*

Y. Shaaltiel, 2008, *"Novel and Biogeneric Protein therapeutics"*. The Biotechnology Day, Meitav, Kiryat Shmone, Israel.

A. Zimran, E.Almon-Brill, **Y.Shaaltiel**, Y.Liberman, D.Bartfeld, G.Galili, D.Aviezer, E.Galun **2006**"A *plant cell expressed recombinant human glucocerebrosidase (prGCD) administrated IV:A phase 1, non-randomized,open label, single dose-escalation safety study in healthy volunteers.* 7th International Workshop European Working Group on Gaucher Disease University of Cambridge, UK.

Y. Shaaltiel,. 2005 *'Expression of biologically active antibodies in an industrial scale plant cell culturing device'* Plant-Based Vaccines & Antibodies (PBVA June 2005) Prague, Czech Republic

Pitcovski, J. **Y. Shaaltiel**, A. Safadie, M. Malkinson & Y. Weismann **1992** *Computerized image analysis of bursae of fabricius of chickens infected with virulent and vaccine strains of infectious bursal disease virus.* The World's Poultry Science Association The 30th annual convention pp. 30.

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N. Cohen, A. Warshawsky, D. Meishar, **Y. Shaaltiel**, J. Gressel.**1990**. *Chelators as synergists to herbicides causing photo-oxidation.* 11th conference of the Weed Science Society of Israel.

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Shaaltiel, Y., Jansen, M., Glaser, A., Gepstein, S., Warshawsky, A., Cohen, N., Chua, N.-H. and Gressel, J. **1988** 10th conference of Weed Science Society of Israel Phytoparasitica 16:4 367-368

Shaaltiel, Y., Jansen, M., Warshawsky, A. and Gressel, J. **1988**. *Chloroplast mode of evolved photo-oxidant resistance in Conzya.* X Int. Congress of Photobiology, Jerusalem.

Gaba, V., Ben-Amotz, A., Cohen, N., **Shaaltiel, Y.** and Gressel, J. **1987**. *Source of photodynamic activity of acifluoren.* X Israel. Convention of Weed Control.

Shaaltiel, Y., Glazer, A., Gepstein, S., Warshawsky, A., Chua, N.H. and Gressel, J. **1987**. *Resistance to paraquat in Conzya: biochemistry, genetics, controlling resistance by synergism.* X Israel Conv. on Weed Control.

Shaaltiel, Y. and Gressel, J. **1987**. *Resistance to environmental and xenobiotic oxidants: appearance, protection and suppression.* Isr. Bot. Soc.

Shaaltiel, Y. and Gressel, J. **1987**. *Copper and zinc chelators synergistic for oxidant generating herbicides.* Weed Sci. Soc. America.

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Shaaltiel, Y. and Gressel, J. **1985**. *Mechanism of paraquat tolerance in Conzya bonariensis.* Plant Physiol. 77s, 162.

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Mizrahi, Y., **Shaaltiel, Y.** and Arad, S. **1980.** *A method for the separation or identification of endopolygalacturanase isozymes from a crude extract of tomato fruit by means of gel electrophoresis.* Isr. Bot. Soc.

Patents and patent applications:

"*Mucosal or enteral administration of biologically active macromolecules* "Shaaltiel, Yoseph , Almon, Einat WO2007010533 EP1904638

"System and method for production of antibodies in plant cell culture Hashmueli, Sharon Shaaltiel, Yoseph Bartfeld, Daniel Baum, Gideon Ratz, Tal Mizrachi, Einat Forester, Yehava WO2006040764 EP1799813,

"Variants of human glycoprotein hormone alpha chain: compositions and uses thereof" - Shemesh, Ronen Shaaltiel, Yoseph Baum, Gideoshalev, Gil Dahary, Dvir Bernstein, Jeanne WO2005044851 EP1682576

"Production of high mannose proteins in plant culture" Shaaltiel, Yoseph Hashmueli, Sharon Bartfeld, Daniel Baum, Gideon Lewkowicz, Ayala WO2004096978 EP1618177

"Cell/tissue culturing device, system and method" Shaaltiel, Yoseph WO2005080544 EP1718726

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"Synergists for herbicidal composition" Israel J. Gressel ,Shaaltiel, Yoseph . IL 77817 WO8704596 EP0258387

"Recombinant Infectious Bursal Disease Proteins and Poultry Vaccines Containing them" Pitkovski, **Y.Shaaltiel**, B. Levi.. IL 108788